

Final Report

INVESTIGATION OF THE TRANSFORMATION OF URANIUM UNDER IRON-REDUCING CONDITIONS: REDUCTION OF U^{VI} BY BIOGENIC Fe^{II}/Fe^{III} HYDROXIDE (GREEN RUST)

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1. RESEARCH OBJECTIVE

The recent identification of green rusts (GRs) as products of the reduction of Fe^{III} oxyhydroxides by dissimilatory iron-reducing bacteria, coupled with the ability of synthetic (GR) to reduce U^{VI} species to insoluble UO₂, suggests that biogenic green rusts (BioGRs) may play an important role in the speciation (and thus mobility) of U in Fe^{III}-reducing environments. The objective of our research was to examine the potential for BioGR to affect the speciation of U under Fe^{III}-reducing conditions. To meet this objective, we designed and executed a hypothesis-driven experimental program to identify key factors leading to the formation of BioGRs as products of dissimilatory Fe^{III} reduction, to determine the key factors controlling the reduction of U^{VI} to U^{IV} by GRs, and to identify the resulting U-bearing mineral phases. The results of this research significantly increase our understanding of the coupling of biotic and abiotic processes with respect to the speciation of U in iron-reducing environments. In particular, the reduction of U^{VI} to U^{IV} by BioGR with the subsequent formation of U-bearing mineral phases may be effective for immobilizing U in suboxic subsurface environments. This information has direct applications to contaminant transport modeling and bioremediation engineering for natural or enhanced *in situ* remediation of subsurface contamination.

2. RESEARCH PROGRESS AND IMPLICATIONS

This report summarizes work after 3 years of a 3 year project. Microbial activity plays a key role in the biogeochemical cycling of Fe in aquatic and terrestrial environments. Indeed, the presence of Fe^{II} in suboxic and anoxic environments is typically attributed to the action of dissimilatory iron-reducing *Bacteria* (DIRB) and *Archae*, a diverse group of microorganisms that are able to couple the oxidation of organic compounds or molecular hydrogen to the reduction of Fe^{III} resulting in the production of a suite of Fe^{II} species consisting of soluble Fe^{II} complexes, surface Fe^{II} complexes on organic and inorganic solid phases, and a host of Fe^{II}-bearing mineral phases, including magnetite (Fe₃O₄), siderite (FeCO₃), vivianite (Fe₃(PO₄)₂), and GRs. Green rusts are mixed ferrous/ferric hydroxides that are found in many suboxic environments and have been shown to reduce a number of organic and inorganic contaminants including chlorinated solvents, nitrate, chromate, and U^{VI}. However, a detailed understanding of the microbial and chemical controls on the formation of GRs and their subsequent reactivity as reducing agents for contaminant transformation has been lacking and is the focus of this project.

2.1 Microbial Factors Contributing to Biogenic Green Rust Formation

Previous studies have reported on the formation of BioGRs from dissimilatory iron reduction of the Fe^{III} oxides (ferrihydrite and lepidocrocite); however, all of these studies were conducted with either *Shewanella putrefaciens* strain CN32 or *Shewanella putrefaciens* strain ATCC 8071. Therefore, it is not clear if the formation of BioGR from dissimilatory iron reduction is unique to *S. putrefaciens* or if it can occur with a broader range of DIRB. *Shewanella* species are a phylogenetically diverse group of bacteria that have been isolated from a wide range of aquatic

and terrestrial environments and exhibit broad versatility with regard to anaerobic respiration. Therefore, at Argonne we examined the potential for BioGR formation as a result of dissimilatory iron reduction of lepidocrocite by a series of *Shewanella* species (*S. alga* BrY, *S. amazonensis* SB2B, *S. baltica* OS155, *S. denitrificans* OS217T, *S. loihica* PV-4, *S. oneidensis* MR-1, *S. putrefaciens* ATCC 8071, *S. putrefaciens* CN32, *S. saccharophila*, and *Shewanella* sp. ANA-3) that together represent much of the phylogenetic diversity within this genus. All of the *Shewanella* species examined (with the exception of *S. denitrificans* OS217T) were able to respire on lepidocrocite, however, there were significant differences in the rates of Fe^{II} production (ranging from $4.5 \text{ mM Fe}^{\text{II}} \text{ d}^{-1}$ for *S. putrefaciens* CN32 to $0.1 \text{ mM Fe}^{\text{II}} \text{ d}^{-1}$ for *S. amazonensis* and *S. baltica*). Despite these differences, GR was the only Fe^{II} -bearing solid phase formed in these systems, as indicated by X-ray diffraction (XRD, Argonne), Mössbauer spectroscopy (University of Iowa (UI)), and scanning electron microscopy (SEM, Argonne). In addition, SEM imaging revealed significant differences in the shapes of the GR particles produced by the various *Shewanella* species. There was no evidence for the formation of other Fe^{II} bearing minerals, such as siderite (FeCO_3) or magnetite (Fe_3O_4), that are commonly observed as products of DIR of Fe^{III} oxides. The formation of BioGR by *Shewanella* species isolated from a wide range of habitats and possessing varied metabolic capabilities suggests that under favorable conditions BioGRs may be formed by a diverse array of DIRB.

We have also determined that the availability of specific electron donors and electron shuttles has a significant effect on the formation of BioGRs during the dissimilatory reduction of Fe^{III} oxides. At Argonne we surveyed the ability of *S. putrefaciens* CN32 to utilize a range of potential electron donors including acetate, citrate, ethanol, formate, glucose, glutamate, glycerol, lactate, H_2 , malate, *N*-acetylglucosamine, pyruvate, serine, and succinate for dissimilatory reduction of lepidocrocite. Of the *e*- donors tested, formate, H_2 , lactate, *N*-acetylglucosamine, pyruvate, and serine supported substantial Fe^{III} reduction. XRD (Argonne) and Mössbauer (UI) analysis of the resulting solids revealed that siderite was the dominant Fe^{II} biomineralization product when serine or pyruvate were provided, while GR was the dominant product with formate, H_2 , lactate, and *N*-acetylglucosamine, indicating that the choice of electron donor provided can control the type of biomineralization product formed. We (Argonne) also determined that the rate of Fe^{III} reduction (and subsequent GR formation) could be enhanced by the presence of a series of potential electron shuttling compounds (chemicals that can facilitate the transfer of electrons from the organism to the insoluble Fe^{III} oxide) including low molecular mass quinones and humic substances. In general, the rate of Fe^{II} production increased with decreasing reduction potential of the given hydroquinone/quinone redox couple.

2.2 U^{VI} reduction by Biogenic Green Rust

In recent years it has been shown that synthetic GRs are able to chemically reduce many organic and inorganic contaminants; in a previous study we reported on the facile reduction of U^{VI} to U^{IV} by synthetic GR resulting in the formation of uraninite nanoparticles. However, differences in the reactivity of synthetic vs. biogenic Fe^{II} phases are often pronounced, which makes it difficult to assess the reactivity of biogenic Fe^{II} phases based on the reactivity of synthetic analogs. At Argonne, we probed the reactivity of BioGRs produced by several *Shewanella* species (*S. putrefaciens* CN32, *S. alga* BrY, and *Shewanella* sp. ANA-3) during the bioreduction of lepidocrocite. We used the X-ray absorption fine structure (XAFS) spectroscopy technique known as extended X-ray absorption fine structure (EXAFS) spectroscopy to measure the chemical speciation of U, thereby allowing us to measure the potential for the reduction of

soluble U^{VI} to insoluble U^{IV} by the BioGRs. U^{VI} , as uranyl chloride, was added to aqueous suspensions of BioGRs that were pasteurized to eliminate the potential for microbial reduction of U^{VI} and washed repeatedly to remove any soluble reductants. After 48 h, loss of U^{VI} from solution was measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) at ANL. For all of the BioGRs examined, > 99% of the added U was removed from solution.

The solids were collected by centrifugation and analyzed by EXAFS spectroscopy at the Materials Research Collaborative Access Team (MRCAT/EnviroCAT) beamline 10-ID at the Advanced Photon Source (APS) at Argonne. The EXAFS data were fit with a model based on the structure of uraninite (UO_2), containing 8 oxygen atoms (O1), 12 uranium atoms (U1), and 24 oxygen atoms (O3), but modified to include contributions from 1-3 Fe atoms and a splitting of the O1 shell. The average number of Fe atoms is consistent with 1 – 3 Fe neighbors per U atom. The U^{IV} appears to be present as small uraninite clusters (containing 2 – 6 U atoms on average) that are likely in close association with an Fe bearing solid (e.g., residual BioGR or a BioGR oxidation product such as magnetite). These results demonstrate that U^{VI} is readily reduced to insoluble U^{IV} by BioGRs produced by several *Shewanella* species.

2.3 Interlayer Anion Effects on U^{VI} reduction by Green Rusts

The structure of GRs consists of alternating positively charged Fe^{II}/Fe^{III} hydroxide layers and hydrated anion interlayers. At UI we examined the effects of various interlayer anions (Cl^- , SO_4^{2-} , or CO_3^{2-}) on the reduction of U^{VI} by GR. The kinetics of U^{VI} uptake were determined in batch reactors containing aqueous suspensions of sulfate, chloride, or carbonate GR (GRSO₄, GRCl, and GRCO₃, respectively). Although there are differences in the extent of uptake, > 90% of the removal of U^{VI} from solution occurred within 50 min for each of the green rusts examined. At Argonne, the solids were collected by centrifugation and analyzed by EXAFS spectroscopy at the MRCAT/EnviroCAT beamline 10-ID at the APS to determine whether U^{VI} is adsorbed and/or reduced by the green rusts. In GRCl and GRSO₄ suspensions > 99% of added U^{VI} is removed from solution with > 90% reduced to U^{IV} . The EXAFS data for the resulting U^{IV} phase are consistent with the formation of single molecules or small molecular clusters of UO_2 . Although > 80% of U added to the GRCO₃ suspensions is associated with the solid phase, there is no indication of significant (> 10%) reduction to U^{IV} . These results clearly indicate that the reactivity of green rusts with respect to the reduction of U^{VI} to U^{IV} is affected by the nature of the interlayer anion.

2.4 Conclusions

We have identified many of the key physiological and chemical factors controlling the rate and extent of BioGR formation from dissimilatory Fe^{III} reduction and demonstrated that BioGRs are highly effective at reducing soluble U^{VI} to insoluble U^{IV} . These results show how the coupling of biotic and abiotic processes can lead to contaminant transformation—specifically with respect to the speciation of uranium and more generally to the speciation of other redox-active contaminants, under Fe^{III} -reducing conditions. This information has direct application to understanding contaminant transport and the development of *in situ* bioremediation technologies for treatment and long-term-stewardship strategies addressing subsurface contamination by uranium (and other redox-active heavy metals and radionuclides).

3. PLANNED ACTIVITIES

This project has now been completed.

4. INFORMATION ACCESS

4.1 Journal Articles and Theses:

- Boyanov, M., E.J. O'Loughlin, M. Scherer, and K. Kemner. (in preparation). Structure of the U(VI)-CO₃ complex responsible for uranyl uptake by carbonate green rust. *Environ. Sci. Technol.*
- Boyanov, M., E.J. O'Loughlin, M. Scherer, and K. Kemner. (in preparation). Edge-sharing U(IV)-Fe complexes resulting from U(VI) reduction by sulfate and chloride green rust. *Environ. Sci. Technol.*
- Boyanov, M., E.J. O'Loughlin, M. Scherer, and K. Kemner. (in preparation). Evidence for ternary Fe- U(IV)-CO₃ association after uranyl reduction by sulfate green rust in the presence of carbonate. *Environ. Sci. Technol.*
- Martin St. Clair, Maxim I. Boyanov, Ken Kemner, Drew Latta, Sharon L. Smith, Justine O. Harrison, Edward J. O'Loughlin, and Michelle M. Scherer. (in preparation). Carbonate Effects on the Kinetics of U(VI) Reaction with Green Rusts. *Environ. Sci. & Technol.*
- O'Loughlin, E.J., D. Lata, R.E. Cook, K.M. Kemner, and M.M. Scherer. (in preparation). Mineralogical controls on the formation of biogenic green rust during the bioreduction of Fe(III) oxides and oxyhydroxides by *Shewanella putrefaciens* CN32. *Geochim. Cosmochim. Acta.*
- O'Loughlin, E.J., S.D. Kelly, and K.M. Kemner. (in preparation). Reduction of U(VI) by biogenic green rust. *Environ. Sci. Technol.*
- O'Loughlin, E.J., P. Larese-Casanova, D. Lata, R.E. Cook, and M.M. Scherer. (in preparation). Electron donor effects on the bioreduction of lepidocrocite by *Shewanella putrefaciens* CN32. *Geomicrobiol. J.*
- O'Loughlin, E.J. (in review). Electron transfer mediator effects on the bioreduction of lepidocrocite by *Shewanella putrefaciens* CN32. *Environ. Sci. Technol.*
- O'Loughlin, E.J., P. Larese-Casanova, R.E. Cook, and M.M. Scherer. (in press). Biogenic green rust formation from dissimilatory iron reduction of lepidocrocite: Comparison of several *Shewanella* species. *Geomicrobiol. J.*
- Smith, Sharon L. (2006). Contaminant Reduction by Fe(II) Species. Ph.D. Thesis, Civil and Environmental Engineering, University of Iowa.
- Justine O. Harrison. (2005) Uranium(VI) Removal in the Presence of Green Rust. M.S. Thesis, Civil and Environmental Engineering, University of Iowa.
- Kemner, K.M., E.J. O'Loughlin, S.D. Kelly, and M.I. Boyanov. 2005. Synchrotron x-ray investigations of mineral-microbe-metal interactions and their effects on metal transformations. *Elements* 1:217-221.
- O'Loughlin, E.J., K.M. Kemner, and D. R. Burris. 2003. Effects of Ag^I, Au^{III}, and Cu^{II} on the reductive dechlorination of carbon tetrachloride by green rust. *Environ. Sci. Technol.* 37: 2905-2912.
- O'Loughlin, E.J., S.D. Kelly, K.M. Kemner, R. Csencsits, and R.E. Cook. 2003. Reduction of Ag^I, Au^{III}, Cu^{II}, and Hg^{II} by Fe^{II}/Fe^{III} hydroxysulfate green rust. *Chemosphere* 53: 437-446.
- O'Loughlin, E.J., S.D. Kelly, R.E. Cook, R. Csencsits and K.M. Kemner. 2003. Reduction of uranium(VI) by mixed Fe(II)/Fe(III) hydroxide (green rust): Formation of UO₂ nanoparticles. *Environ. Sci. Technol.* 37: 721-727.

4.2 Presentations

- O'Loughlin, E. J., K. M. Kemner, M. M. Scherer, M. I. Boyanov, M. St. Clair, S. L. Smith, J. O. Harrison, R. E. Cook, S. D. Kelly, and B. Ravel. 2006. Transformation of uranium under Fe(III)-reducing conditions: Reduction of U(VI) by biogenic Fe(II)/Fe(III) hydroxide (green rust). *Gordon Research Conference/Environmental Sciences: Water*, Plymouth, NH
- O'Loughlin, E. 2006. Transformation of U(VI) under iron-reducing conditions. *DOE-ERSP PI Workshop*, Warrenton, VA.
- O'Loughlin, E. J., M. M. Scherer, K. M. Kemner, S. Kelly, M. Boyanov, B. Ravel, R. Cook, M. St. Clair, S. Smith, and J. Harrison. 2006. Investigation of the Transformation of Uranium under Iron-Reducing Conditions: Reduction of U^{VI} by Biogenic Fe^{II}/Fe^{III} Hydroxide (Green Rust), DOE-ERSP PI Workshop, April, 3-5, Warrenton, VA
- O'Loughlin, E. J., S. Kelly, R. E. Cook, P. Larese Casanova, M. M. Scherer, and K. M. Kemner. 2006. Transformation of uranium under Fe(III)-reducing conditions: Reduction of U(VI) by biogenic Fe(II)/Fe(III) hydroxide (green rust). *231st American Chemical Society National Meeting*, Atlanta, GA.
- St. Clair, M., S. L. Smith, J. O. Harrison, E. J. O'Loughlin, K. M. Kemner, M. I. Boyanov, and M. M. Scherer. 2006. U(VI) reaction with green rusts: Influence of anions. *231st American Chemical Society National Meeting*, Atlanta, GA.
- O'Loughlin, E. J., P. Larese-Casanova, R. E. Cook, and M. M. Scherer. 2006. Comparison of dissimilatory iron reduction of lepidocrocite among *Shewanella* species. *106th General Meeting, American Society for Microbiology*, Orlando, FL.
- Khare, T., E.J. O'Loughlin, S.L. Tollaksen, C.B. Lindberg, N. VerBerkmoes, M. Shah, M. Thompson, R. Hettich, and C.S. Giometti. 2006. Proteomic analysis of *Shewanella oneidensis* MR-1 grown on insoluble iron oxides. *106th General Meeting, American Society for Microbiology*, Orlando, FL.
- O'Loughlin, E., P. Larese Casanova, R. Cook, and M. Scherer. 2005 Biogenic green rust formation from dissimilatory iron reduction of lepidocrocite: Comparison of several *Shewanella* species. *The Joint International Symposia for Subsurface Microbiology (ISSM 2005) and Environmental Biogeochemistry (ISEB XVII)*, Jackson, WY.
- Boyanov, M. I., E. J. O'Loughlin, S. K. Kelly, J. B. Fein, E. E. Roden, and K. M. Kemner. 2005. Reduction of U(VI) by adsorbed vs. surface-precipitated Fe(II) at model cell surfaces. *15th Annual Goldschmidt Conference*, Moscow, ID.
- O'Loughlin, E. J., M. M. Scherer, K. M. Kemner, J. Harrison, M. Boyanov, and S. Kelly. 2005. Investigation of the Transformation of Uranium under Iron-Reducing Conditions: Reduction of U^{VI} by Biogenic Fe^{II}/Fe^{III} Hydroxide (Green Rust), DOE-NABIR PI Workshop, April, 18-20, Warrenton, VA
- Meyer, H. P., E. J. O'Loughlin, D. J. Sholto-Douglas, and K. M. Kemner. 2004. Electron donor effects on the reduction of AQDS by several *Shewanella* species. *104th General Meeting, American Society for Microbiology*, New Orleans, LA.
- Sholto-Douglas, D. J., H. Meyer, M. Donnelly, E. J. O'Loughlin, E. E. Roden, and K. M. Kemner. 2004. Investigation of dissimilatory iron reduction in a mixed culture of *Shewanella oneidensis* and *Geobacter sulfurreducens*. *104th General Meeting, American Society for Microbiology*, New Orleans, LA.
- O'Loughlin, E. J., M. M. Scherer, and K. M. Kemner. 2004. Investigation of the Transformation of Uranium under Iron-Reducing Conditions: Reduction of U^{VI} by Biogenic Fe^{II}/Fe^{III} Hydroxide (Green Rust), DOE-NABIR PI Workshop, March 17-19, Warrenton, VA